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TRANSLATION

DESCRIPTION

METHOD AND DEVICE FOR SELECTIVELY DETECTING FERROMAGNETIC
OR SUPERPARAMAGNETIC PARTICLES.

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The invention relates to the detection of magnetic
particles as markers in analytes.

From the state of the art, detection processes with
analytes by means of fluorochromes, enzymes or radioactive
10   particles as so called markers for analytes are known. It is a
drawback that the linear detection range of fluorescent markers or
the sensitivity of enzymatic techniques are limited. Radioactive
markers are problematical because of the need to provide radiation
protection.

15   Bioassays on the basis of magnetic marking of analytes is
therefore one alternative. The magnetic particles are comprised of
an iron oxide core with a defined diameter of several tens of
nanometers to several hundred nanometers. They have a
biocompatible surface coating with which they can bond to the
20   analytes, for example, to chemical substances, or to the surfaces
of cells or viruses in a manner known per se.

Advantageously, such markers are stable, nontoxic and
manipulatable by means of magnetic fields. Particles of iron
oxide are superparamagnetic. The presence of magnetic particles
25   in a sample volume can be determined by alternating field

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susceptometry.   In the case of monodispersitates, i.e. a unitary particle size, the concentration of the particles can also be quantitatively determined.

From United States Patent 6,110,660 the detection of magnetic particles by means of susceptometry is known. In that case the magnetic susceptibility of an analyte is measured by means of a Maxwell bridge in the frequency range around 200 kHz. The measured electrical voltage on an output amplifier of the bridge is proportional to the susceptability of the solution. With constant particle size, the susceptibility is also proportional to the number of magnetic particles in solution.

The drawback is that this method is not selective. Indeed the magnetic susceptibility of concentrated nanoparticle solutions is high. However, immunoassay methods as a rule detect very small concentrations of biomolecules and consequently very small concentrations of magnetic marker particles. The resulting susceptibility of the solution is then very small and can hardly be distinguished from the susceptibility of a control solution or blank without magnetic particles. To increase the amplification at the output of the Maxwell bridge is hardly a usable solution to this problem because of parasitic effects like susceptibility variations in the sample vessel, the reagents and the laboratory environment as well as the spread in the output voltage give rise like thermal effects and electrical drifting of the components of the readout circuit interfere.

From United States Patent 6,046,585, the movement of
magnetic particle samples to produce a low frequency modulation of
the measurement signal using a qradiometric SQUID magnetic field
detector is known. The drawback here is that signals from the
5   sample holder and the sample vessel cannot be suppressed in this
process.

It is the object of the invention, therefore, to provide
a method for selectively detecting superparamagnetic   particles
and/or ferromagnetic particles which allow such particles to be
10   detected in a highly sensitive manner with low apparatus costs. It
is an object of the invention further to provide a device for
carrying out the method.

The objects are achieved with a method according to the
main claim and a device in accordance with the auxiliary claim.
15   Advantageous features will be apparent from the patent claims
respectively dependent upon them.

For the selective detection and/or for quantifying
superparamagnetic and/or ferromagnetic particles, because of the
nonlinearity of the magnetization characteristics of the particles
20   frequency components of the magnetic fields with a mixed frequency
are measured.   Superparamagnetic and ferromagnetic substances have
a nonlinear magnetization characteristic line which is a
characteristic used in accordance with the invention for the
selective detection of these substances. The method utilizes the
25   dependency of the differential susceptibility (that is the

derivative of the magnetic susceptibility) upon the magnetic field acting on the particles.

The particles are subjected to a first alternating magnetic field.   This has the consequence of modulating the magnetization characteristic line of the particles (modulating magnetic field).

Preferably the modulating magnetic field has a frequency between 50 and 100 Hertz. By selecting such low frequency alternating magnetic fields, low currents and voltages can be used.

In addition, the particles are subjected to a second alternating magnetic field   with a different frequency from that of the modulating magnetic field.   The second alternating field serves for scanning the nonlinearity of the magnetizing field characteristic line of the particles (scanning or exploring magnetic field).

As a consequence, as a result of the effects of the first alternating magnetic fields, there is an induced response magnetic field of the magnetic particles which can be measured.

The scanning magnetic field can advantageously have a frequency between 10 and 100 kilohertz. For this purpose when an induction coil is advantageously used as a magnetic field sensor, a voltage is induced by the response magnetic field in this coil which is proportional to the frequency of the response magnetic field, is relatively high and thus is easily measurable.

The amplitude variation which arises from the response magnetic is primarily dependent upon the type or concentration of

the magnetic particles. Superparamagnetic and ferromagnetic
materials have, as previously indicated, a nonlinear magnetization
characteristic based upon the nonlinear magnetization
characteristic material which can be selectively detected.

5   The response magnetic field has at a sensor mixed
frequency components of the two alternating magnetic fields applied
to the magnetic particles. One such component can be detected by
suitable phase sensitive detection (demodulation). The voltage
thus produced corresponds to the amplitude course of the component

10   and is part of the output signal or is the output signal for
determining the concentration of the analyte.

          It has been found to be especially advantageous to
measure the time course of the amplitude (amplitude variation) of
the response magnetic field by phase sensitive detection of the

15   frequency of the response magnetic field.

          In the case of superparamagnetic and ferromagnetic
analytes, the time course of the amplitude of the magnetic field
will show frequency components which are a multiple of the
frequency of the modulating magnetic field. Especially pronounced

20   are even multiples and especially the component with the double
frequency of the modulating magnetic field.

          The reason for this is that the modulating magnetic field
results in a distorted magnetic induction of the magnetic
particles. The additionally applied scanning magnetic field has,

25   in the case in which the modulating magnetic field has an
instantaneous magnetic induction in a zero pass, the consequence

that a large additional magnetic induction will result. In the
case in which the modulating induction is at a maximum. The
scanning magnetic field by contrast contributes only to a small
additional induction. The amplitude is monitored by the derivative
of the magnetization curve of the magnetic particles which in the
case of large magnetic fields, because of the saturation effect,
is smaller than in the case of smaller magnetic fields. If one
considers the time course of the amplitude of the response magnetic
field to the frequency of the scanning magnetic field as a function
of time, one finds that this amplitude varies directly with twice
the frequency of the frequency of the modulating magnetic field.
It has its minimum at the extremes of modulation and its maximum
at the zero passes.

The amplitude variation of the response magnetic field is
demodulated by phase sensitive detection. The response magnetic
field is converted in a magnetic field sensor to an electric
voltage and optionally is amplified.

The resulting output voltage is in the case of
monodispersed particles, advantageously linearly proportional to
the concentration of the analyte which can be calculated after
calibration of the measurement system.

A device for the selective detection and/or
quantification of superparamagnetic and or ferromagnetic
particles by means of differential of the susceptibility of the
particles comprises a container or vessel for the analyte with the
particles to be detected and/or quantified. The container can be

composed of a nonmagnetic material suitable for the process.   That is, for example, of glass or a synthetic resin (plastic).

The device comprises at least one oscillator. The oscillator is suitable for producing frequencies of alternating

5   magnetic fields as are required for the present case.

Especially advantageously, the device comprises a basis frequency oscillator from which the frequencies of the scanning magnetic field and the modulating magnetic field are derived by means of frequency dividers in a phase-correct relationship.

10   However, also two oscillators can be used for the independent generation of both alternating magnetic fields. A first oscillator then produces the frequency of the scanning magnetic field, a second oscillator the frequency of the modulating magnetic field.

15   The device has at least one field generator. This serves for applying the alternating magnetic fields to the analyte or subjecting the analyte to the alternating magnetic fields. There can especially advantageously be two such field generators provided which subject the analyte to the first or second

20   alternating magnetic field, that is the modulating magnetic field and the scanning magnetic field.

The device comprises a magnetic field sensor which serves to pick up a response magnetic field from the particles. The magnetic field sensor converts and amplifies the amplitude

25   variation of the magnetic response of the particles and supplies to it to a phase sensitive detector for further processing.

The device comprises, in addition, at least one phase sensitive detector which is supplied from an oscillator optionally through a frequency divider with a reference frequency. The phase sensitive detector enables the amplitude of the response magnetic field to be determined   at this frequency.

The device can encompass at least one second phase sensitive detector which is also fed by an oscillator, optionally through a frequency divider, with a reference frequency. In this case, the first phase sensitive detector advantageously supplied with the scanning frequency as the reference, supplies the amplitude of the response magnetic field at the scanning frequency. The second phase sensitive detector is supplied with a reference in the form of a multiple of the frequency of the modulating magnetic field. The second phase sensitive detector searches out the amplitude of the magnetic response field supplied by the first phase sensitive detector to the second phase sensitive detector for a frequency component which is an even number multiple, especially the double of the frequency of the modulating magnetic field. The second phase sensitive detector produces an output voltage corresponding to the amplitude of the frequency component.

To maximize the demodulated signal the device can further have phase shifters at the reference inputs of the phase sensitive detectors.

Below the invention is described in greater detail based on embodiment examples and the accompanying FIGS. 1 through 8.

FIG. 1 diagrammatically illustrates the basic physical characteristics of superparamagnetic and ferromagnetic materials. These materials have a nonlinear magnetization characteristic [magnetization characteristic line] (FIG. 1b). By contrast thereto, diagrammatic and paramagnetic materials have a linear magnetization characteristic (FIG. 1a).

The substances are excited with an alternating field of the frequency f and the amplitude H_a. A static or steady state magnetic field may additionally be applied, for example the Earths magnetic field and is designated H_o.

$$H(t) = H_a \sin(2\pi ft) + H_0 \qquad\qquad (1)$$

FIG. 1a shows the magnetic modulating induction 3 of a paramagnetic substance on a modulating magnetic field 1. A paramagnetic substance is characterized by a constant permeability

$\mu = \mu o$ μr where μr is greater than 1. $\mu_0 = 4\pi \cdot 10^{-7} \dfrac{V_s}{Am}$ The

permeability of the vacuum is given by equation

and μ_r represents the relative permeability count or coefficient of the material. The resulting magnetic modulating induction 3 is given by

$$B(t) = \mu_0 \mu_r H_a \sin(2\pi f t) + \mu_0 \mu_r H_0 \qquad (2)$$

which, as shown in FIG.1a is an unmodified sinusoid. A paramagnetic material with a linear magnetization characteristic is supplied, therefore, an undistorted response. The same applies for diamagnetic materials with a constant relative permeability

μ_r <1.

Superparamagnetic substances have the expected nonlinear and nonhysteretic magnetization characteristics 5. The magnetization M(H) of superparamagnetic substances can generally be described by the formula

$$M(H) = M_S L\left(\frac{\mu_o H}{B_c}\right) \qquad (3)$$

In this equation

$$L(x) = \coth(x) - \frac{1}{x} \qquad (4)$$

which represents the Langevin function, B_c the characteristic indication, M_s the saturation magnetization of the particle and

$$x = \frac{\mu_0 H}{B_C}$$

the argument of the Langevin function.

The typical magnetic response of a superparamagnetic material is shown as a distorted sin function for the magnetic modulation induction 6 in FIG. 1b and is generally described by inserting Formula (1) in Formula (3) as shown in Formula (5):

$$B(t) = M_S L\left(\frac{\mu_o}{B_c} \left[H_a \sin(2\pi f t) + H_0 \right] \right) \qquad (5)$$

According to the invention, a modulating magnetic field 4 is used to modulate the magnetization characteristic 5.   The modulating magnetic field 4 is an alternating magnetic field of a certain frequency f.

5   In addition, the analyte is subjected to a second alternating field (not shown), a so-called scanning magnetic field. The frequency of the scanning magnetic field is selected to be different from the modulated magnetic field 4.

FIG. 2 shows the basic principle utilizing the method.
10   FIG. 2 b describes the case of a superparamagnetic or ferromagnetic analyte and FIG. 2a the case of a diamagnetic or paramagnetic analyte. The modulating magnetic field 4 as was illustrated in FIG. 2, gives rise to a distorted magnetic modulating induction 6 whose time dependency is described by the formula (5). The additional
15   applied second alternating magnetic field gives rise, as a scanning magnetic field, in the case in which the modulating magnetic field generated magnetic modulated induction 6 passes through its zero point, to a large additional scanning induction at the frequency of this scanning magnetic field (FIG. 2, element 9).

20   In the case in which the induction 6 produced by the first alternating magnetic field (modulating magnetic field passes though a maximum), the scanning magnetic field leads only to a small additional induction (FIG. 2b, element 10).

The amplitude 11 of the response magnetic field can be
25   monitored in terms of the derivative of the magnetization characteristic. In the case of superparamagnetic or ferromagnetic

substances, this slope of the magnetization characteristic in the case of larger magnetic fields $|H|$ is significantly smaller than originally at $H = 0$. For superparamagnetic   substances with a magnetization characteristic according to Equation 5 the derivative of the Langevin function gives

$$\frac{dL(x)}{dx} = 1 - \coth(x)^2 - \frac{1}{x^2} \qquad (6)$$

which for large $|x|$ is significantly smaller than in the original with $x = 0$.

If one sets the course of the amplitude 11 of the magnetic induction, that is the response magnetic field at the frequency of the scanning magnetic field as a function of time, one can see that this amplitude varies directly with twice the frequency of the modulating magnetic field 4. It has its minimum at the extremes and its maximum at the zero passages of the modulating magnetic field 4 or the magnetic modulating induction 6.   In the case of a paramagnetic   analyte one obtains an undistorted   magnetic induction 3 from the modulating magnetic field 1 because of the linear magnetization characteristic 2 in FIG. 1.

In that case, one obtains a uniform   amplitude 8 of the additional induction 7 and independently of the scanning magnetic field or the value which   the modulation magnetic field 1 or the modulating induction 3 may have (FIG. 2a, number 7).

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The corresponding amplitude 8 of the magnetic induction at
the frequency of the scanning magnetic field is constant with time.

Apart from the doubled frequency of the frequency of
modulating magnetic field (or the modulating induction 6) the
5   amplitude 11 of the modulating magnetic field 4 comprises at the
frequency of the modulating magnetic field also higher harmonics of
the frequency of the modulating magnetic field 4. At a symmetrical
modulation the modulating characteristic 5, ($H_0 = 0$)
one will find only frequency components with even number multiples
10   of the frequency of the modulating field. If one considers an
analyte to which is additionally applied a static direct field H_0,
one will obtain additional frequency components with uneven
multiples of the frequency of the modulating   magnetic field.

The method has the advantage that only analytes with
15   nonlinear magnetization characteristics will show a time variation
in the amplitude 11 as illustrated in FIG. 2b. Paramagnetic or
diamagnetic substances with linear magnetization characteristics 2
show a constant amplitude 8 of the   magnetic induction of the
frequency of the scanning magnetic field. This selectivity
20   distinguishes   the method of the invention from the methods of the
state of the art which cannot distinguish whether a measured
susceptibility derives from a diamagnetic or paramagnetic material
or a superparamagnetic or ferromagnetic material.

FIG. 3 shows a first device for the selective detection
25   and/or quantification of superparamagnetic and/or ferromagnetic
materials in analytes. The components are so configured that based

upon the nonlinearity of the magnetization characteristic 5 of the
particles, frequency components of the magnetic fields 15 and 18 can
be measured at a mixed frequency.   Required for this purpose are a
suitable vessel 12 and an analyte contained therein in a measurement

5   volume.   The analyte can be formed by a method known per se like a
physisorption reaction, a chemisorption reaction, precipitation,
filtration or extraction and introduced into the vessel 12.   To the
analyte detectable superparamagnetic and/or ferromagnetic particles
are coupled.

10   An oscillator 16 generates the frequency of the modulating
magnetic field 18 which is applied by means of a field generator 17
to the analyte.

An oscillator 13 generates the frequency of a second
alternating magnetic field, the scanning magnetic field 15 which is

15   applied by means of a further field generator 14 to the analyte.

The response magnetic field 19 which is emitted by the
particles or the analyte is converted by a magnetic field sensor20
into an electrical voltage and preamplified.

In addition, the device comprises a first phase sensitive

20   detector 21 to which the frequency of the scanning magnetic field 15
from the oscillator 13 is fed has a reference.   This phase-sensitive
detector 21 outputs the time course of the amplitude (see FIG. 2
elements 11 or 8) of the response magnetic field 19 by the frequency
of the scanning magnetic field 15.   This amplitude 11 or 8 is fed to

25   a second phase-sensitive detector 23 which is supplied by the
oscillator 16 with the frequency of the modulating magnetic field 18

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through an m-fold frequency multiplier 22 as a reference, to detect a frequency component which corresponds to the m-fold of the frequency of the modulating magnetic field with a whole positive number m. Preferably m is selected to be an even number and especially preferably m = 2. The generated output voltage 24 corresponds to the amplitude of this frequency component. A frequency component is measured at a mixed frequency which only arises because of the presence of the nonlinearity in the magnetization characteristics of the particles.

To the extent that the detection chain 19-23 operates linearly, the output voltage 24 is linearly dependent upon the superparamagnetic/ferroparamagnetic moment in the sample volume of vessel 12.

At a constant moment of the individual magnetic particles in the analyte and constant measurement volume, the concentration of the magnetic marker in the sample is proportional to the output voltage. The amplitude of the modulating magnetic field is advantageously so chosen that the analyte will be driven in magnetic saturation. In the case of superparamagnetic particles, whose magnetization characteristic can be described by equation (3), the amplitude of the modulating magnetic field 18 is selected to be of

the order of magnitude of the characteristic field B_c of the superparamagnetic particles used. The curvature of the magnetization characteristic lines (equation 3) follows the basic Langevin function:

$$\frac{d^2 L(x)}{dx^2} = -2\coth(x) \cdot \left(1 - \coth(x)^2\right) - \frac{2}{x^3} \tag{7}$$

with its maximum at x = 1.37. Especially advantageous, therefore, is an amplitude of the modality magnetic field 18 of $\mu_0 H_a = 1,37 B_c$.

The frequency of the modulating magnetic field 18 for modulating the magnetization characteristic 5 is advantageously selected to be comparatively low, for example between 50 Hz and 100 Hz, so that for the generation of such low frequencies, coils with a high turn count and correspondingly low current and voltage, can be used. The scanning magnetic field 15 is selected advantageously to have a higher frequency, for example between 10 kHz and 100kHz. Especially with the use with induction coils the magnetic field sensor 20 a higher frequency of the second magnetic field has the advantage that the voltage 24 induced in the measurement coil 20 and which is proportional to the frequency, will be high. Any static or steady state environmental direct field is advantageously selected to be as small as possible.

FIG. 4 sketches out an alternative device. A quartz
oscillator 25 is used to generate a frequency which is divided by
means of three frequency dividers 26, 27 and 28. The division
ratio of the three dividers 26, 27 and 28 are so selected that they
5   are respectively

$$\frac{1}{\ell}, \qquad \frac{1}{m \cdot n} \qquad \frac{1}{n}$$

with whole positive numbers 1 and n. The whole positive number m
represents the multiple of the modulation frequency which is to be
10   demodulated. Advantageously m will be even and in an especially
advantageous case m will be selected to be equal to 2.

The field generators 17 and 14 produce modulation magnetic
field 18 and the sensing magnetic field 15. The response magnetic
field 19 is picked up by a differential, that is a gradiometric,
15   induction coil serving as the magnetic field sensor. The coil is so
constructed that it is composed of two identically fabricated but
mutually oppositely wound, series-connected partial coils only one
of which is immersed in the analyte in vessel 12. In this manner it
is possible advantageously to hold the parasitic effects of the
20   direct induction of an electric voltage at the scanning frequency as
low as possible so that the amplitude of the scanning magnetic field
15 can be selected to be very high without overdriving the

preamplifier of the magnetic field sensor 20. In this manner an
increase in the sensitivity of the apparatus can be achieved.

In addition the apparatus has a first phase sensitive
detector 21 to which is applied the frequency of the scanning
magnetic field 15 from the frequency divider 26 as a reference and
which outputs the time course of the amplitude (see FIG. 2 elements
11 and 8) of the response magnetic field 19 at the frequency of the
scanning magnetic field 15. These amplitudes 11 or 8 are searched
by a second phase sensitive detector 23 for a frequency component
which corresponds to the m-fold multiple of the frequency of the
modulating magnetic field 18 for whole positive number m. The
reference frequency in this case for the embodiment of FIG. 4 is
supplied directly from a frequency divider 28 of the frequency of
the oscillator 25 instead of from an oscillator as in the example of
FIG. 3 where a frequency multiplier must be used. Advantageously,
in this manner all three frequencies used are coupled in a fixed
phase or phase correct manner so that any possible oscillator drift
will not be significant for a measurement.

After the two phase sensitive detectors 21 and 23 the
output voltage 24 is available as the measurement signal.

FIG. 5 sketches out a further device. A quartz oscillator 25 is used as the frequency base which supplies three frequency dividers 29, 30 and 28. The divider ratios of the three dividers are so chosen that they are respectively

$$\frac{1}{n+m} \qquad\qquad \frac{1}{n(n+m)} \qquad\qquad \frac{1}{n}$$

with whole positive numbers m and n. Here again m indicates the multiple of the modulation frequency. Advantageously m is even and in an especially advantageous manner is selected to be m = 2. The device of FIG. 5 has only one phase sensitive detector 21 which is supplied with the frequency of the oscillator 25 divided by a factor n as the reference. This phase sensitive detector 21 outputs the time course of the same magnetic field component as in the two previously described embodiments of FIGS. 3 and 4.

Experimentally, samples of different concentrations of magnetic particles were made and measured. For that purpose a solution of colloidal iron oxide particles in the nanometer size range was produced and serially diluted with isotonic table salt solution (NaCl). FIG. 6 shows the measured output voltage with standard deviation as a function of the iron concentration of the samples.

FIG. 7 shows how the measured signal of a sample of fixed concentration varies with a function of a static environmental direct field H_o. Maximum output voltage is obtained at a minimum direct field with a direct field amplitude of about 1.9 mT one observes a minimum of the output voltage. For higher direct fields the signal increases further. From the point of view of the mathematics, the signal follows the third derivative of the Langevin function

$$\frac{d^3 L(x)}{dx^3} = -2(1 - \coth(x)^2)^2 + 4\coth(x)^2 \cdot (1 - \coth(x)^2) + \frac{6}{x^4} \qquad (8)$$

In FIG. 7 this function is compared with the scaled amplitude and matched characteristic field value B_c = 1.4 mT (x = $\mu_o H / B_c$). The agreement with measured values is found to be especially good for small static direct field H_o.

FIG. 8 shows the measured dependency of the output voltage of a sample of fixed concentration in dependence upon the amplitude of the modulating magnetic field. By integration over the amplitude dependent signal course, the signal was calculated and plotted in FIG. 8. The agreement with the measured value is especially good for low amplitudes.

The method/device can be used especially for the following
purposes:

Detection of chemical substances, cells or viruses with
respect to the quantitative determination of the molecular
5   interaction, especially the Ligand-receptor interaction, the
antigen-antibody interaction and interaction between
oligonucleotides whereby superparamagnetic and/or ferromagnetic
particles are coupled to the substances, cells or also viruses.

Reference Character List

1, 4, 18	Modulating magnetic field
2	Magnetization characteristics, linear
3	Magnetic modulation induction for linear magnetization characteristics
5	Magnetization characteristics, nonlinear
6	Magnetic modulation induction for nonlinear magnetization characteristics
7	Additional scanning induction with linear magnetization characteristics
8	Amplitude of the magnetic induction at the frequency of the scanning magnetic field with linear magnetization characteristics
9, 10	Additional scanning induction with nonlinear magnetization characteristics for zero passages (9) and extremes (10).
11	Amplitude of the magnetic induction at the frequency of the scanning magnetic field with nonlinear magnetization characteristics
13, 16, 25	Oscillator
14, 17	Field generator
15	Scanning magnetic field
19	Response magnetic field
20	Magnetic field sensor
21, 23	Phase sensitive detectors

24   Output voltage

26, 27, 28,   Frequency dividers

29, 30